Role of nitric oxide signaling in endothelial differentiation of embryonic stem cells.

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Public Summary:
Signaling pathways that govern embryonic stem cell (ESCs) differentiation are not well characterized. Nitric oxide (NO) is a potent vasodilator that modulates other signaling pathways in part by activating soluble guanylyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP). Because of its importance in endothelial cell (EC) growth in the adult, we hypothesized that NO may play a critical role in EC development. Accordingly, we assessed the role of NO in ESC differentiation into ECs. Murine ESCs differentiated in the presence of NO synthase (NOS) inhibitor NG-nitroarginine methyl ester (L-NAME) for up to 11 days were not significantly different from vehicle-treated cells in EC markers. However, by 14 days, L-NAME-treated cells manifested modest reduction in EC markers CD144, FLK1, and endothelial NOS. ESC-derived ECs generated in the presence of L-NAME exhibited reduced tube-like formation in Matrigel. To understand the discrepancy between early and late effects of L-NAME, we assessed the NOS machinery and observed low mRNA expression of NOS and sGC subunits in ESCs, compared to differentiating cells after 14 days. In response to NO donors or activation of NOS or sGC, cellular cGMP levels were undetectable in undifferentiated ESCs, at low levels on day 7, and robustly increased in day 14 cells. Production of cGMP upon NOS activation at day 14 was inhibited by L-NAME, confirming endogenous NO dependence. Our data suggest that NOS elements are present in ESCs but inactive until later stages of differentiation, during which period NOS inhibition reduces expression of EC markers and impairs angiogenic function.

Scientific Abstract:
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